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## The Effect of Scalp Tissue on Current Shunting during Anodal Transcranial Direct Current Stimulation (TDCS)

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The Effect of Scalp Tissue on Current Shunting during Anodal transcranial Direct  
Current Stimulation (tDCS)

A thesis submitted in partial fulfillment of the requirements  
for the degree of  
Master of Science

By

MARK PATRICK JACKSON  
B.S., Clemson University, 2009

2015  
Wright State University

WRIGHT STATE UNIVERSITY  
GRADUATE SCHOOL

August 14, 2015

I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION  
BY Mark Patrick Jackson ENTITLED The Effect of Scalp Tissue on Current Shunting during  
Anodal transcranial Direct Current Stimulation (tDCS) BE ACCEPTED AS PARTIAL  
FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

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## ABSTRACT

Jackson, Mark Patrick. M.S. Department of Neuroscience, Cell Biology, and Physiology, Wright State University, 2015. The Effect of Scalp Tissue on Current Shunting during Anodal transcranial Direct Current Stimulation (tDCS).

Transcranial Direct Current Stimulation (tDCS) has been used to treat various mental and neurological illnesses. Rodent models have been used to examine physiological changes in the brain after tDCS, as well as to develop safety standards. However, most animal tDCS studies implant an electrode on the brain, potentially altering the path of current during stimulation. Additionally, no studies have been completed specifically examining maximum safe anodal tDCS limits, and a pilot study conducted to determine an electrode montage to examine biological changes of learning and memory from anodal tDCS indicated brain lesion was occurring before a commonly cited lesion threshold of 142.9 A/m<sup>2</sup>. Therefore, the goal of this study was to examine both the effects of anodal tDCS and the rodent's scalp on current shunting during anodal tDCS *in vivo*. Anodal tDCS was applied to the skull of 35 anesthetized male Sprague-Dawley rats for 60 minutes after they were divided into groups either receiving stimulation with an electrode on the skull or scalp tissue. Within each skull and scalp electrode placement group, rats were further separated into groups by tDCS current intensity ( $\mu$ A) received, which was: sham (n=4), 150  $\mu$ A (n=4), 300  $\mu$ A (n=4), 500  $\mu$ A (n=3), 1,000  $\mu$ A (n=4), and 2,500  $\mu$ A (n=3) for the skull electrode placement group. For the scalp electrode placement groups, only stimulations that induced lesion during the skull electrode stimulation were chosen: sham

(n=2), 500  $\mu$ A (n=3), 1,000  $\mu$ A (n=3), and 2,500  $\mu$ A (n=3). Brain lesion was quantified using an Olympus BX-63 microscope with Q100 Blue Camera and CellSens software, which showed brain lesion during skull electrode placement first occurring at 500  $\mu$ A, having a lesion volume of 0.168 mm<sup>3</sup>. At 1,000  $\mu$ A and 2,500  $\mu$ A, the average brain lesion within groups was 6.363 mm<sup>3</sup> and 13.013 mm<sup>3</sup>, respectively. Stimulation of the scalp showed no brain lesion at any of the stimulation groups, suggesting the scalp tissue shunts a portion of the current, and as a result, has different physiological effects on brain lesion development.

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## I. INTRODUCTION

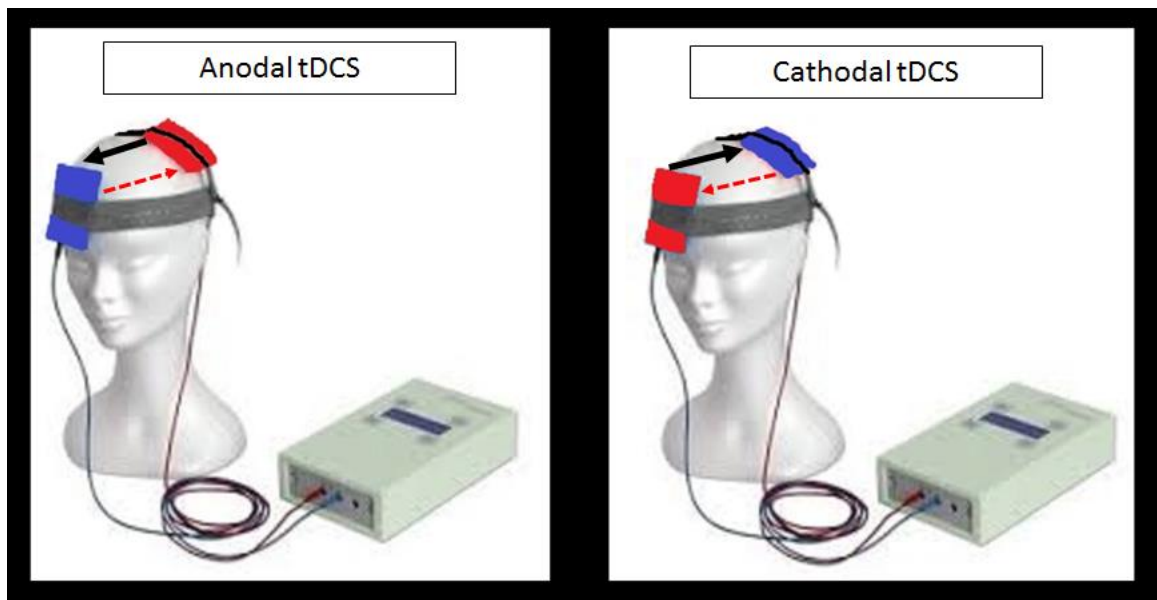
### **tDCS: Background**

Transcranial Direct Current Stimulation (tDCS) is a noninvasive method of electrical brain stimulation and has been used both clinically and experimentally to treat multiple brain disorders and diseases. Electrical stimulation has been used as a medical treatment for a long time; ancient Egyptians and even Plato and Aristotle have recorded the benefits of electrical stimulation (Kellaway, 1946). Modernized forms of electrical stimulation began in the early 20<sup>th</sup> Century, when Electro-convulsion Therapy (ECT) was used to treat mental health disorders such as depression (Lehmann, 1965) and schizophrenia (Tanowitz, Rappa, & Marchitello, 1959). Eventually, ECT gained a negative public reputation as being an unsafe treatment method (Kalayam & Steinhart, 1981), allowing alternative methods of electrical stimulation, such as tDCS, to be refined. Today, tDCS is considered a safe method of treatment despite few studies being conducted on the safety of tDCS. Additionally, *in vivo* tDCS studies using rodents typically attach electrodes to the skull, while human tDCS experiments place the electrode on the scalp tissue. Scalp tissue has been shown to decrease the amount of current reaching the brain while also spreading the current to brain regions further away from the electrode. This study indirectly looks at the effects of skin tissue on tDCS current by measuring brain lesion with two electrode placement locations: scalp and skull tissue.

Many scientists have contributed to the development of tDCS, but research really began to increase when Nitsche and Paulus (2000) were able to show weak direct currents can alter cortical excitability both during and after the stimulation period (Nitsche & Paulus, 2000). These experiments showed tDCS could potentially be a viable, safe, and inexpensive option for clinical physicians treating patients with various neurological disorders (Dubljevic, Saigle, & Racine, 2014). Research has since provided evidence that tDCS in humans can have positive effects on mental health disorders such as major depressive episodes (Meron, Hedger, Garner, & Baldwin, 2015), schizophrenia (Agarwal et al., 2013), and bipolar disorder (Pereira Junior Bde et al., 2015) among others (Tortella et al., 2015). In addition to the medical benefits, other researchers have provided evidence that tDCS can enhance learning and memory in normal, healthy adults (Bennabi et al., 2014). Despite the multiple benefits seen during tDCS research, less work has been done on tDCS safety parameters.

One safety study was conducted by Liebetanz et al. (2009) using one of the two types of tDCS stimulation, cathodal tDCS; the other type of tDCS stimulation is anodal tDCS. The two types of stimulation are determined by which electrode (anode or cathode) is placed over the desired stimulation area (Figure 1). In a tDCS circuit, electrons always move in a loop from the cathodal (positively charged) end of a battery, through the brain and its extracerebral tissues, then back into the anodal (negatively charged) end of the battery (Li & Ke, 2011). To perform anodal stimulation, the anode is placed over the brain region to be stimulated, while cathodal stimulation requires the cathode be placed over the brain region. During anodal stimulation, electrons meet the area of interest under the anode after passing through the brain and its extracerebral

tissues via the cathode. Conversely, when using cathodal stimulation, electrons immediately flood the area of interest, which then flow to the anode. Of note, when discussing electrical current in general, it is common to discuss the current as flowing in the opposite direction of the electrons. Put more simply, electrons always flow from cathode to anode, yet the current is always discussed as flowing from anode to cathode, regardless of the orientation of the electrodes (Wagner et al., 2014).



**Figure 1:** Differences between Anodal and Cathodal tDCS. Electric currents (solid black line) are described as flowing from anode (red electrode) to cathode (blue electrode); however, electrons flow (red dashed line) from cathode to anode. To induce anodal stimulation, the anode is placed over the desired area of stimulation. Conversely, the cathode is placed over the area of stimulation for cathodal tDCS.

## **tDCS Today: Neurologic, Mental Health, and Learning/Memory Enhancement**

Today, researchers and clinicians alike use both tDCS stimulations for many reasons, ranging from improving function in people with neurological diseases to enhancing learning and memory abilities in humans. In clinical settings, tDCS is used to treat a wide variety of neurological diseases, such as stroke (Gomez Palacio Schjetnan, Faraji, Metz, Tatsuno, & Luczak, 2013), some types of neuropathy (Kim et al., 2013), and Autism deficits (Amatachaya et al., 2014). Typically, this is done with anodal stimulation, although cathodal stimulation benefits have been noted (Monti et al., 2013). Regarding stroke, tDCS appears to reduce the severity of signs and symptoms related to the damage resulting from a stroke (Baker, Rorden, & Fridriksson, 2010; O'Shea et al., 2014), although it should be noted tDCS does not entirely diminish or cure the negative effects a stroke or other diseases produce. Pain symptoms resulting from various types of neuropathy have also been alleviated with tDCS treatment (Ngernyam, Jensen, Auvichayapat, Punjaruk, & Auvichayapat, 2013), and promising benefits of tDCS on autism, even years after birth, have also been seen (Amatachaya et al., 2014).

While clinical benefits for neurological problems are beginning to emerge, most tDCS research has been done for relief of various mental health issues. Mental health problems such as depression (Oliveira et al., 2013), bipolar disorder (Pereira Junior Bde et al., 2015), and some neurodegenerative diseases (Elder & Taylor, 2014) have all responded favorably after tDCS treatment. Interestingly, tDCS seems to reverse the negative effects of most mental illnesses treated, regardless of the symptoms. For example, patients with depression treated with tDCS showed improved depression rating scores (Valiengo, Bensenor, Lotufo, Fraguas Jr, & Brunoni, 2013), while patients with

Alzheimer's showed marked improvement in recognition memories after tDCS stimulation (Ferrucci et al., 2008).

In addition to improving memory in patients with Alzheimer's, tDCS studies focusing on improving learning and memory in normal, healthy adults have increased (Matzen, Trumbo, Leach, & Leshikar, 2015; Pope, Brenton, & Miall, 2015). In addition, research on tDCS with attention during monotonous tasks has shown marked improvement in attention when the subjects were sleep deprived compared to Sham or caffeine consumption (McIntire, McKinley, Goodyear, & Nelson, 2014). In addition to attention enhancement, studies on enhancing sports performance with tDCS has shown promising results (Zhu et al., 2015), as well as improving fine motor coordination in musicians with dystonia (Furuya & Altenmuller, 2015). The beneficial effects of tDCS seen in normal, healthy individuals is an exciting new area of research. However, because tDCS as a whole is a fairly new approach, caution should be exercised before it is approved for use on the general public.

### **General Issues Moving Forward: Ethics, Stimulation Localization, and Public Perception**

From a biochemical perspective, little is known regarding how tDCS actually results in changes to the brain's structure (de Berker, Bikson, & Bestmann, 2013), and while studies of tDCS function are beginning to increase, many are being performed on humans where inconclusive data prevents researchers from moving to Phases III and IV (Brunoni et al., 2012). This raises issues that tDCS researchers are beginning to address, such as safety and efficacy. To understand how prevalent these concerns are, a survey of

tDCS researchers was conducted, and over half of human tDCS first and senior authors that participated expressed doubts regarding both the efficacy and ethics of tDCS (Riggall et al., 2015).

Another interesting issue of ethics and tDCS is that only beneficial effects have been almost exclusively reported. Besides minor skin tingling in humans (Fertonani, Ferrari, & Miniussi, 2015), there have been very few reported unwanted effects. One of the few negative reports showed potentially cytotoxic calcium levels after repeated anodal DC stimulation (Islam, Aftabuddin, Moriwaki, Hattori, & Hori, 1995), highlighting the need for an increased understanding of the physiological effects of tDCS.

To research how tDCS affects the brain, a basic understanding of where the current is moving in the brain should be addressed. Many researchers are studying how tDCS flows through the brain using computational models (Galletta et al., 2015; Parazzini, Fiocchi, Liorni, & Ravazzani, 2015), but *in vivo* models have yet to be fully utilized. During tDCS in humans, electrodes are placed on the scalp tissue, which has been shown to shunt (disperse the electrical current from the direction it was moving) a portion of the current (Faria, Hallett, & Miranda, 2011; Truong, Magerowski, Blackburn, Bikson, & Alonso-Alonso, 2013), making it difficult to determine the concentration and spread of electrical current reaching the brain *in vivo*.

As noted above, less physiological studies of tDCS *in vivo* have been performed compared to clinical studies, and as a result, physiological effects of the stimulation have not been adequately addressed. Also, because clinical studies focus on improving a specific issue, public perception has been mostly positive, even though how tDCS

actually invokes changes in the brain is less understood. The lack of negative effects from tDCS suggests to the public that tDCS is completely safe, but caution should be observed before agreeing with the public's positive perception of tDCS (Dubljevic et al., 2014). Many drugs with no known side effects prescribed to the general public without sufficient research have resulted in severe, unpredicted side effects, such as with Thalidomide (Miller & Stromland, 2011). It would be beneficial to all invested in tDCS to understand any negative effects, and this would be more safely done utilizing models other than humans.

### **Computational Modeling and Animal Preparations in tDCS**

There are two common types of models used for tDCS research: computational and both *in vivo* and *in vitro* animal preparations. Computational models use existing knowledge of how electricity acts on tiny, isolated areas of the brain, such as a neuron, and combines that information to predict how the current acts on the brain as a whole (Wagner et al., 2014), which is done using different types of computational modeling. One common type of computational modeling used in tDCS is Finite Element Modeling (FEM), which incorporates mathematical equations into a representative model made of materials that represent the brain's cellular properties (Laakso & Hirata, 2012). tDCS is then applied to the brain model, and different aspects of the current's effect on the brain can be measured (Rush & Driscoll, 1968; Truong et al., 2013).

FEM is usually studied via computer software, and can involve simple (Faria et al., 2011) or complex (Saturnino, Antunes, & Thielscher, 2015) representations of the brain and its surrounding tissues. Additionally, the finite aspects of brain



electrodynamics can be combined to create a more homogenous mathematical representation to study tDCS effects (Dougherty, Turner, & Vogel, 2014). These models are invaluable to tDCS research, but the greatest understanding of tDCS current can be realized when computational modeling is combined with animal preparations.

Animal preparations are the other commonly used method of tDCS research, with the rodent (mouse and rat) being a common type of animal used (Bennabi et al., 2014; Bolzoni, Baczyk, & Jankowska, 2013). Utilizing animals can be a powerful tool for testing *in vivo* effects of tDCS, however, as with most animal preparations, there are some limitations for translating animal research findings to human physiology. Despite the limitations when using animals to test the effects of tDCS, a great amount of insight has been gained to the underlying neural mechanisms affected by tDCS (Pedron, Monnin, Haffen, Sechter, & Van Waes, 2014; Rahman et al., 2013). However, there are some differences when delivering a tDCS current in humans that are not used with rodents, one of which being the electrode montage used.

### **Translational Considerations: Electrode, Montage, and Tissue**

There are many different types of electrodes available for tDCS (Bolzoni, Pettersson, & Jankowska, 2013; McIntire et al., 2014; Pedron et al., 2014), and each type of electrode is constructed using different materials. Using only one type of electrode could stunt progress, but the variations make choosing an electrode for tDCS more difficult because different electrodes have different conductive properties and materials. Further, the electrodes used in rodent models are specially designed for rodents

(Liebetanz et al., 2006), and are not representative of tDCS electrodes used during humans studies.

Typically, animal studies utilize a type of electrode jacket that is implanted onto the skull and filled with a conducting solution that an electrode is then screwed into (Liebetanz et al., 2009; Pedron et al., 2014), whereas human studies utilize sponge electrodes soaked in a conductive material placed on the scalp (DaSilva, Volz, Bikson, & Fregni, 2011). In addition, different electrode montages are beginning to be explored, such as the newly emerging HD-tDCS. This high definition electrode montage has been suggested to improve the localization of stimulation (DaSilva et al., 2015), and is beginning to be used in humans.

Another issue limiting animal translation is the effect electrode placement has on electrical currents in the brain. This problem arises due to differences in head size between rodents and humans (Takano et al., 2011). The distance between anode and cathode has been shown to affect how the current travels (Faria et al., 2011), ultimately dictating which regions of the brain a current reaches. A smaller distance between anode and cathode has been shown to increase the amount of current shunting, resulting in a less focused current distribution during stimulation (Datta, Elwassif, Battaglia, & Bikson, 2008; Faria et al., 2011; Rush & Driscoll, 1968). Further, placing both electrodes on the head results in both anodal and cathodal stimulation, termed bilateral stimulation. Bilateral stimulation can have undesired effects in human tDCS if the montage was not intended, and using bilateral tDCS would be difficult to replicate on rodents due to head size constraints.

Another component to consider when choosing an electrode montage is the orientation of neurons at the stimulation site. Computational research has shown when the current is directed downward to a neuron, an effect will occur; if the current is directed to the side of a neuron, however, a different effect occurs. Additionally, other effects are seen when a tDCS current hits a neuron at other angles (Bikson & Rahman, 2013; Rahman et al., 2013). The precise angle a current moves through a neuron cannot be known with *in vivo* models; heads come in different shapes and sizes, but computational models can be controlled for different sizes and shapes of individual tissue *in vivo* during stimulation (Truong, Magerowski, Pascual-Leone, Alonso-Alonso, & Bikson, 2012), giving insight to how electrodes should be placed in live models.

In addition to electrode type and montage, another translational issue to consider is where to place the electrode in rodent tDCS, which is generally implanted surgically onto the skull to maximize the amount of current reaching the rodent's brain (Bolzoni, Baczyk, et al., 2013; Liebetanz et al., 2006; Liebetanz et al., 2009). Stimulating with the electrode on the skull tissue presents a significant translational inconsistency due to human tDCS using scalp electrodes, where most of the electrical current is believed to be shunted away from the brain (Faria et al., 2011). Further, the individual properties of the scalp tissue has been shown to affect the portion of the current that does reach the brain (Truong et al., 2013), indicating rodent tDCS models are receiving more current in the brain region being stimulated. Using an electrode montage on scalp tissue during rodent tDCS would enhance the ability of rodent studies to translate to human physiology during tDCS by mimicking the scalps effects.

Liebetanz et al. (2009) studied safe tDCS stimulations in rodents using brain lesion during cathodal tDCS at varying intensities to determine the maximum safe dosage before lesion was observed. Currents utilized were between 1 and 1000  $\mu\text{A}$  for stimulation durations between 3.33 and 270 minutes with an electrode of 3.5  $\text{mm}^2$ . They were able to show brain lesion occurs during a cathodal stimulation with a current intensity of 500  $\mu\text{A}$  lasting at least 10 minutes, resulting in a current density of 142.9  $\text{A}/\text{m}^2$ . Additionally, a current of 1000  $\mu\text{A}$  lasting at least 3.33 minutes also induced lesion in the rat. Furthermore, they showed a correlational relationship between charge density [calculated as: (Current Intensity \* seconds of stimulation)/electrode size] and brain lesion to arrive at a lesion threshold of 54,000  $\text{C}/\text{m}^2$ . Despite not testing anodal tDCS, they suggested the lesion parameters would be very similar to cathodal tDCS lesion limits (Liebetanz 2009).

## **Conclusion and Hypothesis**

The effects of scalp shunting have not yet been tested *in vivo*, but from computational models studying the effects of scalp tissue on the current during tDCS (Faria et al., 2011; Truong et al., 2013), it can be predicted that tDCS using rodents with an electrode placed on the scalp will result in less current reaching the brain. Additionally, it has been thought that brain lesion would occur at the same current density for both anodal and cathodal tDCS (Liebetanz et al., 2009), while others have been more conservative regarding safety similarities between the two types of tDCS currents due to a lack of anodal tDCS lesion data (Bikson, Datta, & Elwassif, 2009). Interestingly, a rodent pilot study performed in our lab was conducted to determine an appropriate anodal tDCS montage to study the neurophysiology of learning and memory

showed brain lesion occurring at current densities lower than the 142.9 A/m<sup>2</sup> threshold determined by the Liebetanz et al study.

Therefore, the purpose of this study was twofold: 1) test the brain lesion threshold of 142.9 A/m<sup>2</sup> during cathodal tDCS during anodal tDCS in rodents when an electrode is implanted on the skull, and 2) indirectly study the decrease in current reaching brain tissue from scalp shunting during tDCS by comparing differences between brain lesion when the electrode is placed on the skull and scalp. The results of these experiments should enhance our understanding of tDCS safety as well as the ability to use animal preparations for translation to human physiological changes resulting from tDCS.

## II. METHODS

All procedures were approved by the Wright-Patterson Air Force Base (WPAFB) Institutional Animal Care and Use Committee (IACUC) and performed in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011). The experiments were performed on 35 male Sprague-Dawley rats (Charles River, Wilmington, MA), weighing 300-500 grams, and split into two groups by electrode placement: skull (n=21) and scalp (n=14). Animals were double housed under standard laboratory conditions, including a 12 hour light/dark cycle with food and water available *ad libitum*. Following a ten day quarantine/acclimation period, the skull electrode stimulation animals underwent surgery to place an electrode on the skull. After surgery, animals were singly housed and allowed to recover at least 7 days prior to inclusion in any experiments. Following surgery, animals were randomly placed into six anodal tDCS treatment groups: Sham, (n = 3), 150  $\mu$ A (n = 4), 300  $\mu$ A (n = 4), 500  $\mu$ A (n = 2), 1,000  $\mu$ A (n = 4), and 2,500  $\mu$ A (n = 3). Scalp stimulation animals were randomly placed into 3 anodal tDCS groups that indicated lesion present during stimulation with a skull electrode, plus a sham group (n = 4): 500  $\mu$ A (n = 4), 1,000  $\mu$ A (n = 3), and 2,500  $\mu$ A (n = 3).

## **Skull Placement Surgery**

Animals were anesthetized with isoflurane (Piramal Critical Care, Shop Med Vet, Mettawa, IL) using 5% induction and 2-3% maintenance. Animals were treated with standard pre- and post-surgical care, according to WPAFB vivarium IACUC approved Standard Operating Procedure's (SOP's). The animal was placed into a stereotaxic apparatus and a caudo-rostral incision was made on top of the head, then a lateral incision was made at the shoulders. The periosteum was removed, the skull was wiped clean, and a head electrode of 25 mm<sup>2</sup> (ValuTrobe, Axelgaard Manufacturing Co., Fallbrook, CA, 1.25 inch diameter electrode cut to 5mm x 5mm) was applied to the skull with the center of the electrode resting on the midline 2.5 mm caudal to bregma (rostrocaudal: 0.0 mm to -5.0 mm). The insulated electrode wire was tunneled subcutaneously and exited the incision made at shoulders. The electrode was held in place by a plastic head clamp which caught on the ridges of the skull (AFRL designed and produced) and two types of adhesives. C&B Metabond Adhesive Luting Cement (Parkell Inc., Edgewood, NY) was applied to the electrode and skull to create an initial bond, followed by acrylic cement (Stoelting, Co. Fisher Scientific, Pittsburg, PA) to bond the electrode to the clamp. Incisions were then closed around cement and wire by suture. Animals recovered from surgery for at least 1 week prior to inclusion in experiments.

## **tDCS Treatment**

Animals were anesthetized with isoflurane (Piramal Critical Care, Shop Med Vet, Mettawa, IL), using a 5% induction and 2-3% maintenance schedule. The reference electrode (8.04 cm<sup>2</sup>, ValuTrobe, Axelgaard Manufacturing Co., Fallbrook, CA) was

placed between the shoulders and Signagel electrode gel (Parker Laboratories, Fairfield, NJ) was used as the conductor. A Petflex cohesive bandage (Andover, Shop Med Vet, Mettawa, IL) was wrapped around the midsection of the animal's torso to hold the reference electrode in place. tDCS was applied using a constant current stimulator (Magstim DC-stimulator, Neuroconn, Ilmenau, Germany) for 60 minutes. The animals receiving sham stimulation were also anesthetized, had the reference electrode placed, and had wires connected to the electrodes, but no current was passed through the electrodes. During skull stimulation, the animals that received the highest dose of stimulation received tDCS at the highest level that the stimulator could provide based on the voltage limitations (5,000  $\mu$ A). Throughout the highest intensity stimulation, the stimulator automatically shut off when the resistance limit was reached. Following the automated shut down, the device was restarted and stimulation resumed at a lower intensity level. The total duration remained at 60 minutes, but the intensity varied during stimulation from 5,000  $\mu$ A to 2,000  $\mu$ A for the highest intensity group. The average intensity for the 60 minute duration for this group was 2,500  $\mu$ A and is referred to as the 2,500  $\mu$ A condition throughout this manuscript. During scalp stimulation, the highest stimulation received was 2,500  $\mu$ A and was decreased by 500  $\mu$ A if the voltage limit was reached; it is also referred to as the 2,500  $\mu$ A group.

### **Histological Processing and Analysis**

Immediately following the cessation of stimulation, animals were euthanized by Euthasol injection followed by exsanguination; tissue was fixed and preserved using intracardiac perfusion using PBS followed by 4% paraformaldehyde was completed. After perfusion, brains were removed and placed into a 4% PFA solution for twenty-four



hours then transferred to a 30% sucrose solution for two days until the brains sank. Brains were kept in solution at 4° C until processed. A sliding microtome with a freezing plate (Leica SM2010R) was used for serial collection of 16-micron thick sections across the brain. Slices were placed into a cryoprotectant solution and stored at -20 ° C until further processing.

### **H&E Staining**

Two sets of brain sections from each animal were removed from cryoprotectant solution, washed 5 times for 5 min each in 1X PBS and mounted onto slides using a 0.1X PB solution with 5% gelatin. Tissue sections were stained with hematoxylin and eosin (H&E), and images were collected after staining using an Olympus BX63 microscope with a camera (Blue Camera) designed for CellSens Dimension software.

### **Image and Data Analysis**

Researchers quantifying lesion were blinded to treatment groups throughout tissue processing, image collection, and analysis. Individual tissue sections from each animal were placed in rostral-caudal (all tissues were within a range of +5mm Bregma and -6.5 mm Bregma) order using a Stereotaxic Rat Atlas (Paxinos & Watson, 2007), then the area of brain lesion was quantified for each tissue section using CellSens software. All sections fell within a range of +5 mm Bregma and -6.5 mm Bregma, and 0.5 mm increments were chosen as data points to ensure a uniform reporting method along the entire range.

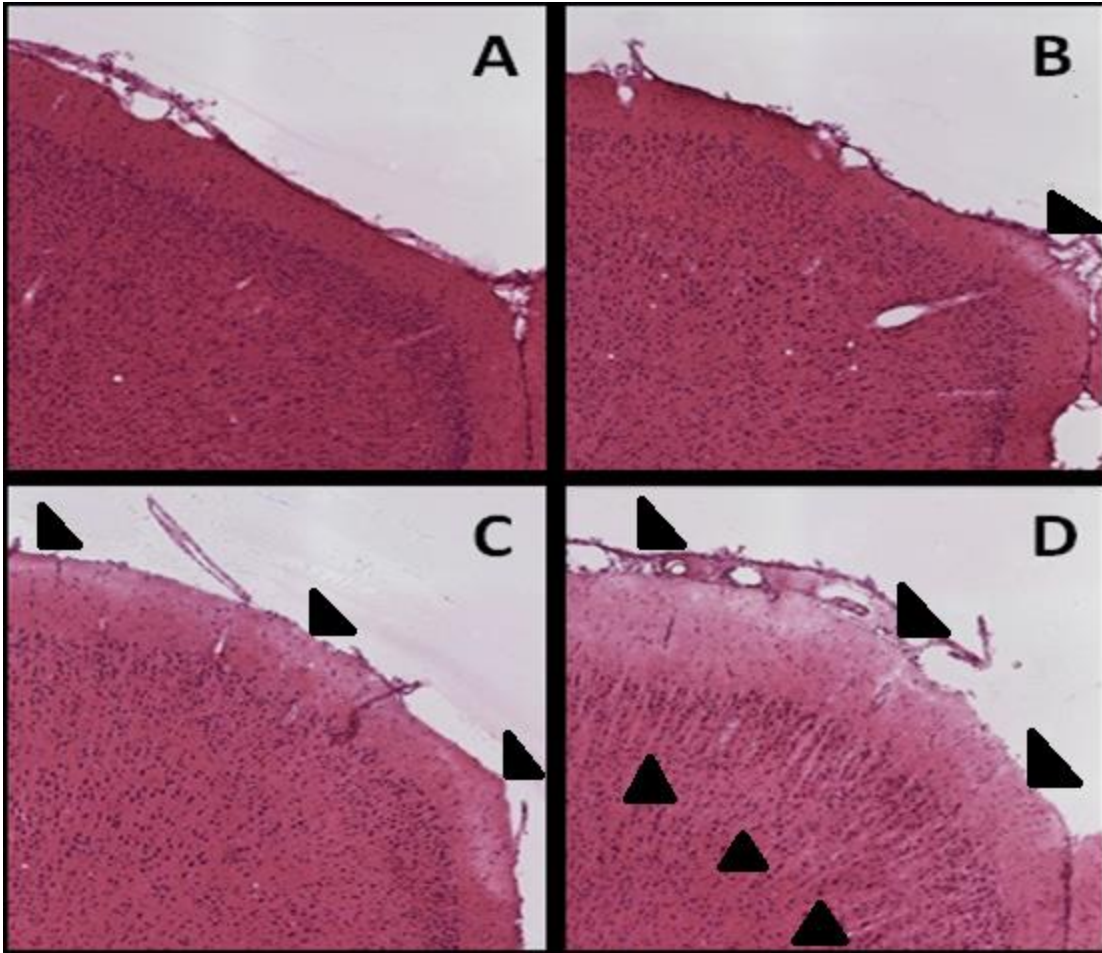
Tissue sections were then averaged to each 0.5 mm increment if they were within 0.4 mm of that data point. The lesion area of each tissue section was averaged together at

its assigned increment, and the sum of all increments gave a total lesion area for each animal. Lesion volume was determined by multiplying the average area at each increment by 0.5 mm. The tDCS current applied to each animal was quantified in multiple ways using the following measurements: current (I), current density ( $I/\text{mm}^2$ ), charge density ( $(I \cdot t)/\text{mm}^2$ ), and Joules ( $I^2 R t$ ), where I is represented in Amperes (A),  $\text{mm}^2$  represents the surface area of the electrode used, t represents time (sec), and R represents the resistance during the stimulation (ohms).

### III. RESULTS

#### **Skull Electrode tDCS Results**

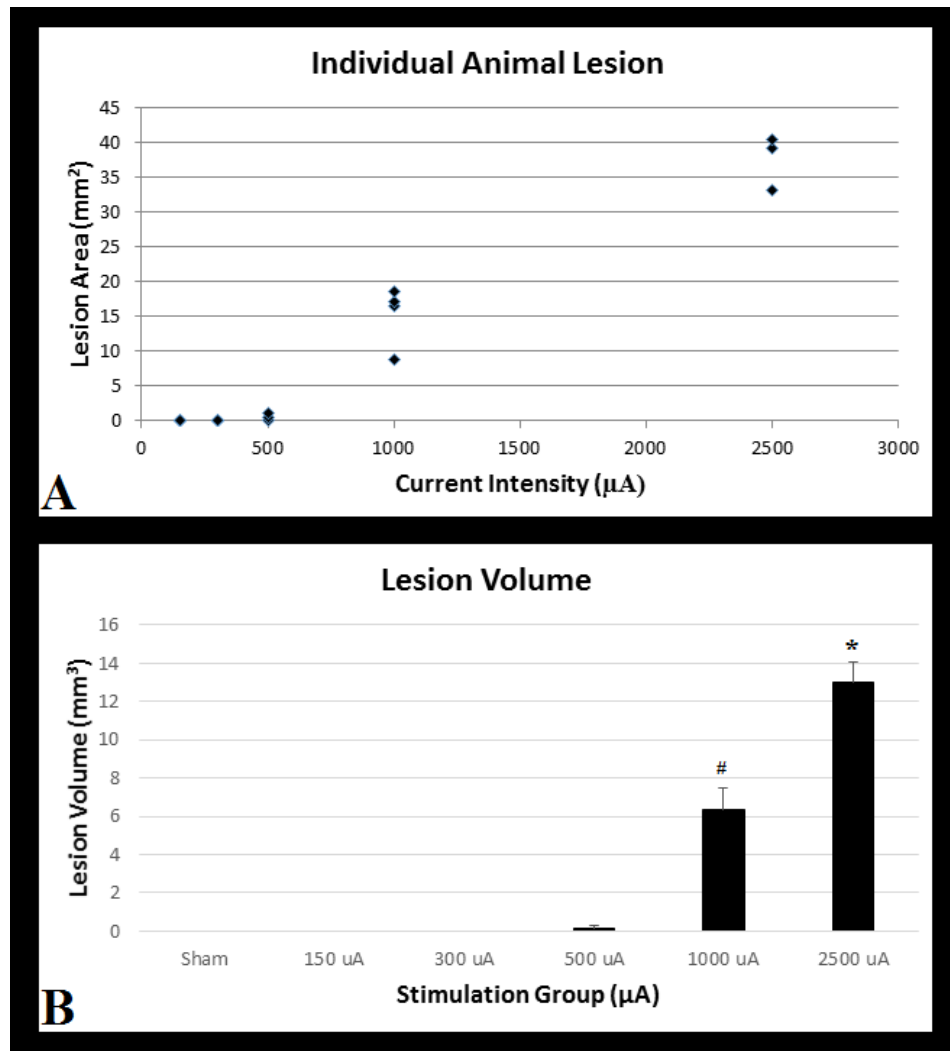
Tissue sections were analyzed for areas of brain lesion by comparing stimulated tissues to the Sham condition. Neither the Sham, 150  $\mu$ A, nor 300  $\mu$ A groups showed brain lesion, while the 500  $\mu$ A, 1,000  $\mu$ A, and 2,500  $\mu$ A stimulation groups did have lesions present (Figure 2). The 500  $\mu$ A group shows a small discoloration at the medial cortex area that does not penetrate deeper than the outer surface of the rat cortex (Fig. 2B). Compared to the 500  $\mu$ A group, the 1,000  $\mu$ A group's discoloration penetrates further into the cortex and spreads laterally across the outer cortex (Fig. 2C). The 2,500  $\mu$ A stimulation group appears to have the greatest area of brain tissue lesion, but the discoloration did not penetrate past cortical brain regions (Fig. 2D).



**Figure 2:** *tDCS-induced brain lesion near -2.5 mm Bregma. Increasing tDCS current increases brain lesion in the rat compared to the Sham (A). A 500  $\mu$ A current (B) induces brain lesion beginning in the medial outer cortex, which spreads laterally and penetrates into the cortex as the current is doubled to 1,000  $\mu$ A (C). This lesion expands laterally and deeper into the cortex when the current is increased to 2,500  $\mu$ A (D). The black arrowheads indicate areas of lesion.*

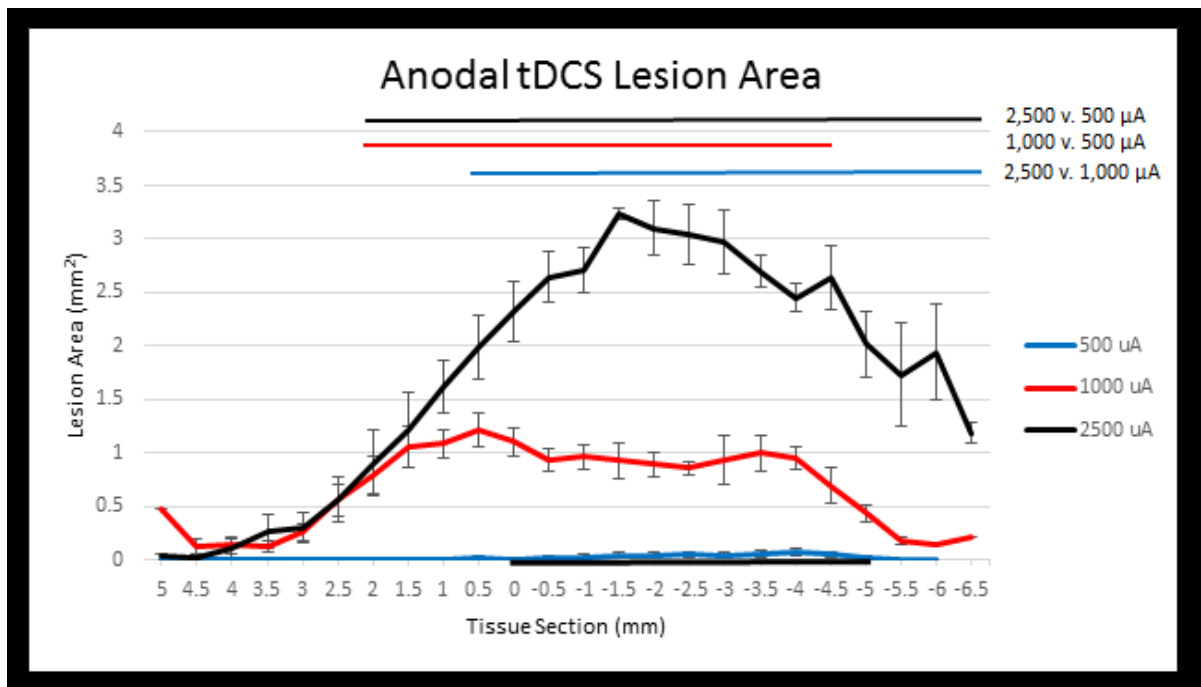
Brain lesion was quantified for all animals by measuring and averaging the volume of brain tissue damage for each stimulation group (Figure 3). Brain damage was present at 500  $\mu\text{A}$  (average volume =  $0.168 \text{ mm}^3$ , SE = 0.105), 1,000  $\mu\text{A}$  (average volume =  $6.363 \text{ mm}^3$ , SE = 1.104), and 2,500  $\mu\text{A}$  (average volume =  $13.013 \text{ mm}^3$ , SE = 1.068) (Fig. 3B).

A one sample t test was performed to compare lesions within stimulation groups to their sample means, which showed no significant differences (Fig. 3A). Using a one way ANOVA, the 2,500  $\mu\text{A}$  stimulation group had a significant volume of lesion compared to all other groups ( $p < 0.001$  for all stimulation group comparisons) groups. Similarly, the 1,000  $\mu\text{A}$  stimulation group had a significant difference compared to all other groups ( $p < 0.001$  for all stimulation group comparisons) (Fig. 3B). There were no other significant differences when comparing the Sham, 150  $\mu\text{A}$ , 300  $\mu\text{A}$ , or 500  $\mu\text{A}$  groups (Fig. 3B).



**Figure 3:** Individual and group lesion quantification. Lesion area was quantified in H&E stained sections obtained from all animals (A) at each stimulation group after tDCS with an electrode implanted on the skull. Average lesion volume was also quantified for each stimulation group (B). In both figures, no lesion was present at currents less than 500 µA, but lesion was seen at currents of 500 µA and greater. \* indicates the 2,500 µA group had significant differences from all other stimulation groups. # indicates the 1,000 µA group had significant differences from all other stimulation groups.

Average brain lesion per tissue section was also analyzed via a two-way ANOVA followed by a Tukey test. (Figure 4). After correcting the p-value for multiple comparisons, no significant differences between stimulation groups appeared until +2 mm Bregma, which occurred between stimulation groups until -6.5 Bregma and are detailed in Table 1. Briefly, significant lesion differences between 2,500  $\mu$ A and 500  $\mu$ A stimulation groups were seen from +2 mm Bregma to -5.5 mm Bregma, and between 2,500  $\mu$ A and 1,000  $\mu$ A stimulation groups ( $p < 0.01$  for all), significant differences range from +0.5 mm Bregma to -5.5 mm Bregma ( $p < 0.05$  for all). Significant differences range from +2 mm Bregma to -5 mm Bregma between 1,000  $\mu$ A and 500  $\mu$ A stimulation groups ( $p < 0.02$ ).



**Figure 4:** Average lesion area by brain tissue section. The average brain lesion was quantified in 0.5 mm increments, with positive numbers indicating the distance rostral to Bregma, and negative numbers indicating the distance caudal to Bregma. The black line at the bottom of the figure indicates the location of the electrode. Significant lesion differences between the lesion inducing currents are indicated at the top of the figure. The bars at each tissue section represent the Standard Error for each stimulation group.

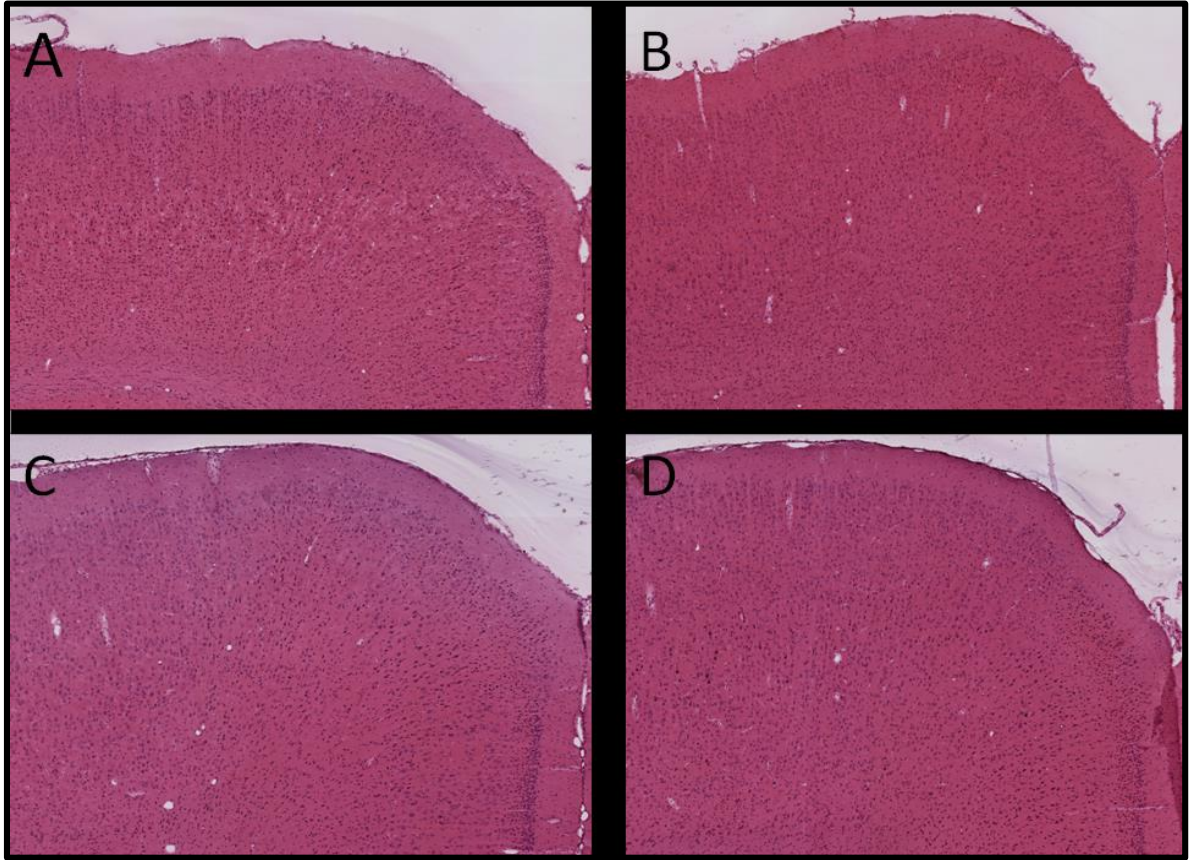


Significant Tissue Section Lesion Differences			
Mean Difference (mm <sup>2</sup> ), p-value			
Tissue Section	2,500 $\mu$ A vs. 500 $\mu$ A	2,500 $\mu$ A vs. 1,000 $\mu$ A	1,000 $\mu$ A vs. 500 $\mu$ A
+2 mm Bregma	0.911, < 0.005	-	0.805, < 0.008
+1.5 mm Bregma	1.217, < 0.001	-	1.058, < 0.001
+1 mm Bregma	1.615, < 0.001	-	1.075, < 0.001
+0.5 mm Bregma	1.967, < 0.001	0.777, < 0.011	1.190, < 0.001
0 mm Bregma	2.305, < 0.001	1.212, < 0.001	1.093, < 0.001
-0.5 mm Bregma	2.624, < 0.001	1.710, < 0.001	0.915, < 0.003
-1 mm Bregma	2.685, < 0.001	1.745, < 0.001	0.940, < 0.002
-1.5 mm Bregma	3.195, < 0.001	2.277, < 0.001	0.918, < 0.003
-2 mm Bregma	3.059, < 0.001	2.208, < 0.001	0.851, < 0.005
-2.5 mm Bregma	2.989, < 0.001	2.179, < 0.001	0.810, < 0.007
-3 mm Bregma	2.922, < 0.001	2.030, < 0.001	0.892, < 0.003
-3.5 mm Bregma	2.638, < 0.001	1.694, < 0.001	0.944, < 0.002
-4 mm Bregma	2.368, < 0.001	1.496, < 0.001	0.872, < 0.004
-4.5 mm Bregma	2.584, < 0.001	1.943, < 0.001	0.641, < 0.05
-5 mm Bregma	2.001, < 0.001	1.579, < 0.001	-
-5.5 mm Bregma	1.730, < 0.001	1.551, < 0.001	-
-6 mm Bregma	1.438, < 0.001	1.323, < 0.001	-
-6.5 mm Bregma	0.791, < 0.017	0.717, < 0.020	-

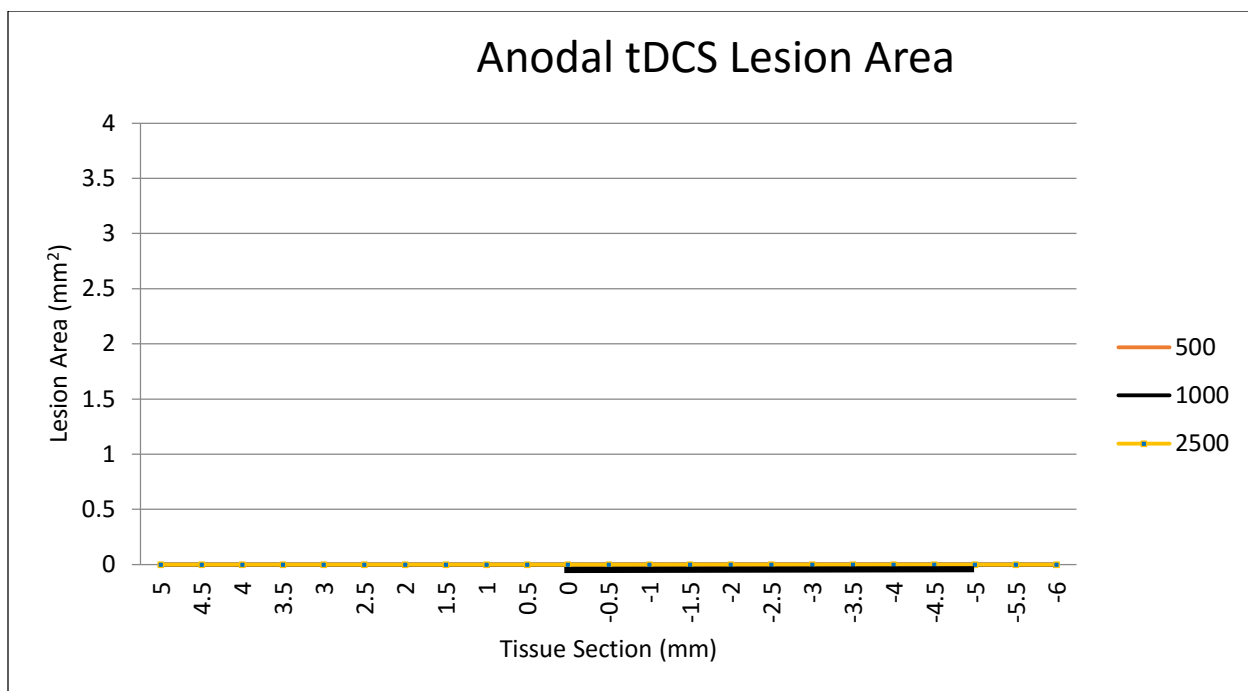
**Table 1:** Specific mean lesion area differences between lesion-inducing currents for each tissue section. For each comparison, the average difference in mm<sup>2</sup> is noted first, followed by the corresponding p-value. The black boxes indicate no significant differences at the tissue section for the comparison.

### Scalp Electrode tDCS Results

Histological analysis of the animals receiving tDCS with the electrode placed on the scalp was performed, and no lesion resulting from anodal tDCS was noted (Figure 5). Further histological analysis did not show tDCS-induced lesion at any tissue section (Figure 6).



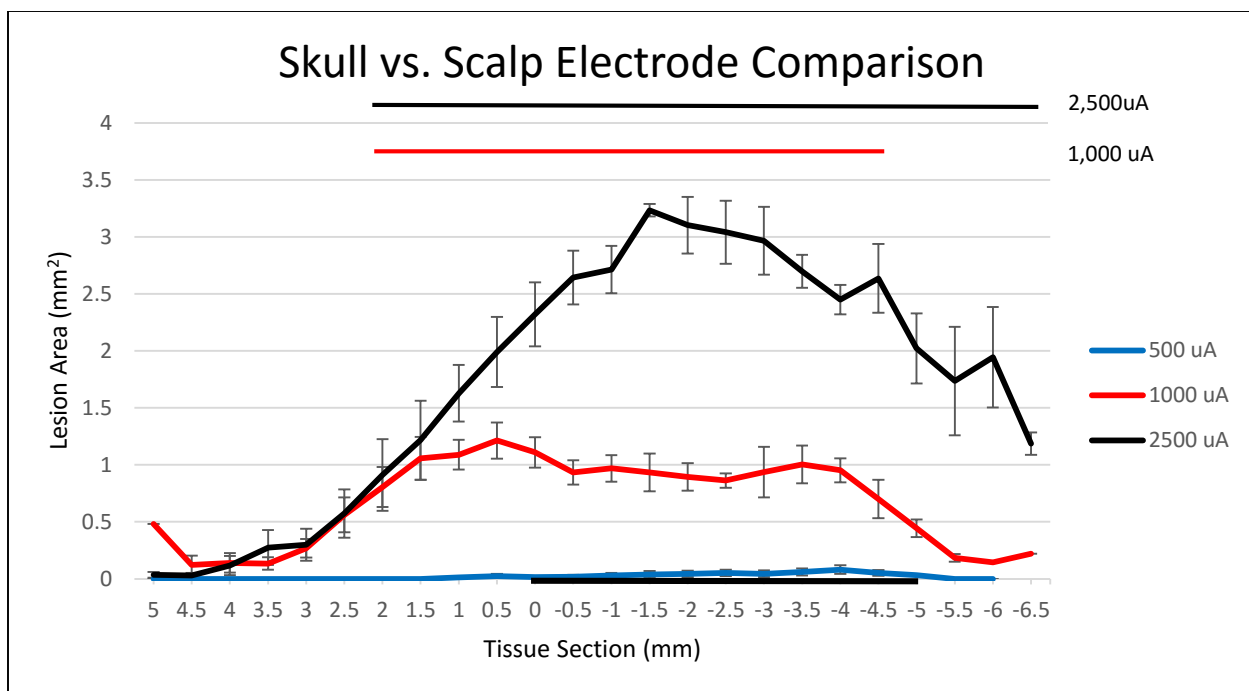
**Figure 5:** Images of brain tissue located near -2.50 mm Bregma after tDCS with an electrode implanted on the scalp. The three stimulation groups are compared to the Sham (A). For all three stimulation groups, no brain lesion was seen at 500  $\mu$ A (B), 1,000  $\mu$ A (C), or 2,500  $\mu$ A (D).



**Figure 6:** Lesion area by tissue section after tDCS with an electrode implanted on the scalp. No lesion was noted at any tissue section for any of the stimulation groups. The black line at the bottom of the figure indicates the placement of the electrode.

### Electrode Placement Comparison: Skull vs. Scalp

Lesion results from each tested stimulation group were compared for significant differences between scalp and skull electrode placement. A three-way ANOVA (corrected for multiple comparisons) was performed to compare the effects of electrode placement, tissue section, and stimulation intensity to lesion. Significant differences in lesion from electrode placement on the scalp and skull at each tissue section are as follows:  $p < 0.001$  from +2 mm Bregma to -5.5 mm Bregma for the 2,500  $\mu\text{A}$  stimulation group, and  $p < 0.001$  from +2 mm Bregma to -4.5 mm Bregma for the 1,000  $\mu\text{A}$  stimulation group. There were no significant differences between scalp and skull electrode placement for the 500  $\mu\text{A}$  group (Figure 7). Specific lesion differences between scalp and skull electrode placement during tDCS are shown in Table 2.



**Figure 7:** A lesion comparison between an electrode implanted on the skull and an electrode placed on the scalp within each stimulation group. The solid black line at the bottom indicates the position of the electrode, and the bars at the top reflect the significant differences between lesion at each tissue section, mm, from tDCS with an electrode placed on the scalp and an electrode placed on the skull. Significant differences are detailed in Table 2.

<b>Skull vs. Scalp Electrode Placement</b>			
Mean Difference (mm <sup>2</sup> ), p-value			
<b>Tissue Section</b>	<b>2,500 <math>\mu</math>A</b>	<b>1,000 <math>\mu</math>A</b>	<b>500 <math>\mu</math>A</b>
2 mm Bregma	0.911, < 0.001	0.805, < 0.001	-
1.5 mm Bregma	1.217, < 0.001	1.058, < 0.001	-
1 mm Bregma	1.628, < 0.001	1.089, < 0.001	-
0.5 mm Bregma	1.990, < 0.001	1.214, < 0.001	-
0 mm Bregma	2.321, < 0.001	1.109, < 0.001	-
-0.5 mm Bregma	2.643, < 0.001	0.934, < 0.001	-
-1 mm Bregma	2.714, < 0.001	0.969, < 0.001	-
-1.5 mm Bregma	3.234, < 0.001	0.957, < 0.001	-
-2 mm Bregma	3.103, < 0.001	0.895, < 0.001	-
-2.5 mm Bregma	3.041, < 0.001	0.863, < 0.001	-
-3 mm Bregma	2.967, < 0.001	0.936, < 0.001	-
-3.5 mm Bregma	2.697, < 0.001	1.004, < 0.001	-
-4 mm Bregma	2.449, < 0.001	0.953, < 0.001	-
-4.5 mm Bregma	2.636, < 0.001	0.693, < 0.001	-
-5 mm Bregma	2.023, < 0.001	-	-
-5.5 mm Bregma	1.735, < 0.001	-	-
-6 mm Bregma	1.438, < 0.001	-	-
-6.5 mm Bregma	0.791, < 0.001	-	-

**Table 2:** Significant differences between skull and scalp electrode placement. Differences between the average lesion at each tissue section are reported. Boxes with dashes represent no significant differences between skull and scalp electrode placement at that tissue section.

#### IV. DISCUSSION

These experiments show anodal tDCS produces lesion on the rodent brain at a current intensity of 500  $\mu\text{A}$  and above when the electrode is placed on the skull, while lesion is not observed at currents between 500  $\mu\text{A}$  and 2,500  $\mu\text{A}$  when the electrode is placed on the scalp. In addition, the area of brain lesion is higher in tissue sections located under the electrode and the degree at which lesion is induced depends on the amount of current injected. Translationally, our rat model with the electrode placed on the skin suggests human tDCS models are well within the lesion safety limits, with human tDCS sessions generally using current densities well below (generally about 10  $\text{A}/\text{m}^2$  or less) our maximum current density tested (100  $\text{A}/\text{m}^2$ ). However, brain damage associated with functional changes at a molecular level during sub-lesion currents was not examined in this study and should be studied more extensively to gain a better understanding of tDCS as it relates to safety.

##### **Anodal tDCS Safety Limits in the Rat**

Our results show brain lesion in rats can be induced with a 500  $\mu\text{A}$  current, corresponding to a current density of 20  $\text{A}/\text{m}^2$ , and the volume of lesion increased as the current was increased (Fig. 3B), showing anodal tDCS at or above 500  $\mu\text{A}$  for durations longer than 60 minutes will induce brain lesion in our rodent model. Because the lesion noted in our study was relatively small at 500  $\mu\text{A}$ , stimulating for durations less than 60 minutes at 500  $\mu\text{A}$  may not produce lesion and warrants further examination. While most rat studies using skull electrodes typically do not stimulate for

durations lasting longer than 30 minutes (Bolzoni, Baczyk, et al., 2013; Pedron et al., 2014), our data can be a valuable guideline for future studies aimed at increasing the amount of current given to the rat to produce various biological effects.

### **Current Localization and Brain Lesion**

The focality of tDCS in the brain can be seen by examining images of brain lesion. Histological analysis (Fig. 1) shows lesion beginning in regions closest to the CSF and spreading laterally and penetrating deeper into the tissue as the current is increased, suggesting the concentration of current in the brain increased with the current. Translating to human tDCS, the current may be affecting the outer cortical layers more than other brain regions, and secondary effects are triggered in other brain regions. Interestingly, some human tDCS studies seem to provide evidence for this, as there is an enhanced benefit from tDCS by activating the brain regions during stimulation, such as when learning a coordinated movement (Zhu et al., 2015).

Physiologically, our brain lesion is seen most in regions under the electrode (Fig. 3), suggesting not only the highest concentrations of current in the brain are near the CSF, but that in addition the greatest concentration is in the CSF under the electrode. This can potentially be explained by considering electricity is able to flow in areas with a lower resistance and is less able to flow in areas that are more resistant, as is the case with a common wire; a low resistance metal is insulated by a high resistance rubber. For tDCS, the conductance properties of four tissues are typically studied: the scalp, skull, CSF, and brain (Datta, Elwassif, & Bikson, 2009). The skull is the least conductive material, while the scalp and CSF are the most conductive (Datta et al., 2009).

It can be inferred from our knowledge of current and conductance that as electrons move towards the anode via the CSF during anodal stimulation, their movement is impeded by the resistive properties of the skull, resulting in more electrons clustering under the electrode as they are not able to move as freely across the skull tissue. The movement of electrons has not been shown *in vivo*, however, an increase in brain tissue pH during and after tDCS in humans has been shown in the area of stimulation (Rae, Lee, Ordidge, Alonzo, & Loo, 2013). Rae et al., 2013 attributed the rise in pH to “activation of the creatine kinase system according to the reaction: Creatine + ATP  $\leftrightarrow$  PCr + ADP + H<sup>+</sup>”, where the extra proton is used to hydrolyze phosphocreatinine, resulting in an increased pH (Rae et al., 2013). While their data does provide evidence of the creatine kinase system for the increased pH under the anode, it is also likely the rise in pH is in part due to increased electrons from the current during anodal tDCS stimulation.

### **Effects of Electrode Placement on the Scalp and Skull**

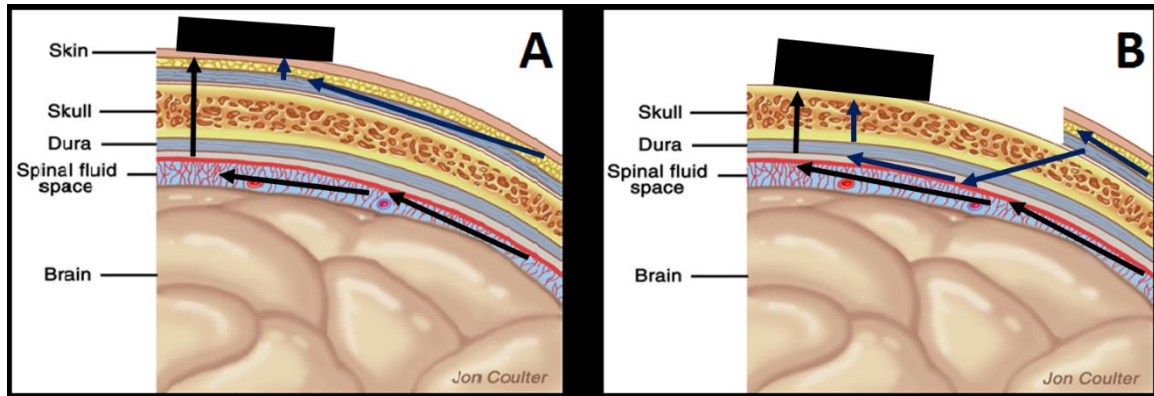
While tDCS was able to induce lesion when the electrode was placed on the skull, there was no lesion present when the same tDCS current was used with the electrode placed on the scalp (Fig. 6). This effect was expected, as computational studies have predicted the scalp shunts the delivery of electrical currents by as much as 50 % (Faria et al., 2011; Truong et al., 2013), and as much as 65% of the current when all tissues between the electrode and brain are considered (scalp, skull, CSF). This study shows scalp shunting diverts enough current to prevent brain lesion at the highest stimulation intensity possible for our model, which was limited for both groups due to reaching maximum voltage limits. Interestingly, 50% of our highest stimulation group (2500  $\mu$ A) with a scalp electrode corresponds to 1250  $\mu$ A, which is greater than two of the three



stimulation groups showing lesion with an electrode placed on the skull. Additionally, 65% of our highest stimulation corresponds to 875  $\mu\text{A}$ , which is still higher than the 500  $\mu\text{A}$  lesion was first seen with a skull electrode, suggesting there may be other interactions involved in current shunting and warrants further study.

It has been previously noted that many researchers study biological changes from tDCS using rodents by implanting an electrode on the skull, while researchers using humans study tDCS by placing the electrode on the scalp. This study indirectly shows there are differences in the concentration of current reaching the brain in an animal model when the electrode is placed on the scalp, suggesting physiological differences resulting from tDCS with a skull implanted electrode may not translate when the electrode is placed on the scalp. Why the current differs with an electrode placed on the scalp could be explained by considering the movement of electrons are following the path of least resistance.

There have been no previous studies examining the differences in current flow between the two electrode placements, but a possibility is that during skull tDCS, the CSF provides the easiest path to the anode from the cathode because the anode is fixed onto the skull, whereas the scalp is the easiest pathway for the current and can get to the anode via the scalp tissue without having to cross between the resistive skull tissue (Figure 8). If true, the different pathways each type of stimulation would explain the differences in brain lesion using the same current. Notably, possible damage to the scalp during stimulation with an electrode placed on the scalp was not evaluated. Additionally, at sub lesion currents, using an electrode implanted on the skull can be expected to have different effects on brain tissue than would be seen using a scalp electrode.



**Figure 8:** Proposed effect of electrode placement on electron movement during anodal tDCS. A theoretical model predicting the flow of current during tDCS. The black box in the figures represent an electrode implanted on the scalp (A) and placed on the skull (B). The arrows represent the predicted flow of most electrons during stimulation, with black arrows representing electron flow under the skull and blue arrows representing electron flow over the skull

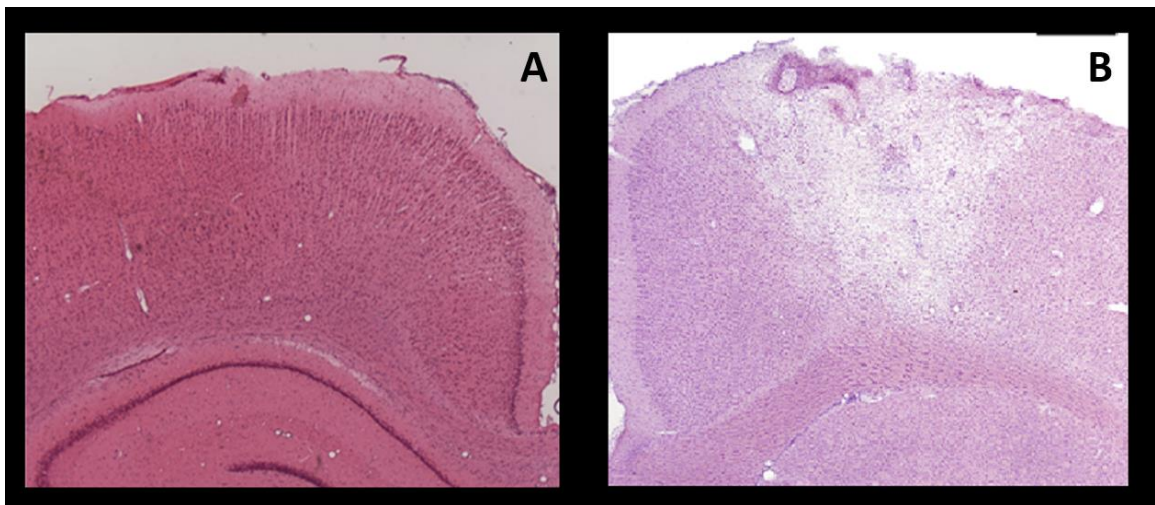
## **Anodal and Cathodal tDCS Comparison**

Comparing our study of anodal tDCS safety with Liebetanz's 2009 study of cathodal safety shows both similarities and differences between the currents. First, a histological comparison (Figure 9) shows cathodal currents induce lesion in a focused, conical shape (Fig. 9B), whereas anodal currents produce lesion closer to the outer cortex and spread more laterally (Fig. 9A). A reason for the difference could be methodological; our electrode was 25 mm<sup>2</sup>, while the electrode used in the Liebetanz et al study measured 3.5 mm<sup>2</sup>. Another methodological issue is the electrode in our study was placed over the center of the brain, beginning at Bregma, during anodal tDCS, while the cathodal stimulation placed the electrode 1.5 mm rostral to Bregma and 1.5 mm to the right of the sagittal fissure. The electrode size and location do seem to prevent any meaningful comparison between our study and the Liebetanz study, but unpublished data using a smaller electrode of 5.31 mm<sup>2</sup> 2.5 mm caudal Bregma and 2.5 mm right of the sagittal fissure during anodal stimulation shows the same patterns of a more lateral and superficial lesion when compared to the lesion during cathodal stimulation.

One possible explanation for the differences in lesion could be deduced from our knowledge of the differences in the movement of current during anodal and cathodal stimulation. The electrons during cathodal stimulation move from the skull to the CSF where the electron flows then moves towards the anode. The anode in Liebetanz's study was placed on the chest, pulling the current down through the brain toward the anode, causing the conical lesion. During anodal stimulation, the electrons left the cathode and were being pulled towards the anode via the CSF, but were impeded possibly because the

anode was implanted on the skull, resulting in a buildup of electrons and a flatter lesion pattern.

The increase in the amount of lesion present during anodal stimulation could have occurred due to an increase in the amount of current within the brain. Because anodal stimulation moves electrons to the anode, most electrons could be predicted to move through the brain before reaching the anode. Conversely, during cathodal stimulation, electrons could travel to the anode in tissues present before reaching the brain. The above explanation of lesion formation is a prediction from our knowledge of anodal and cathodal currents, the veracity of which requires further examination.



**Figure 9:** Comparison of anodal (A) and cathodal (B) brain lesion. Anodal brain lesion is more lateral compared to cathodal stimulation. In addition, cathodal stimulation induced brain lesion that penetrated further into the cortex. The image of anodal brain lesion was taken from our study at a stimulation of 2,500  $\mu\text{A}$ , and the cathodal lesion image is from the Liebetanz et al. (2009) study at 1,000  $\mu\text{A}$ .

### **Previously Proposed Mechanisms of Lesion**

Three proposed mechanisms of electrical stimulation responsible for inducing brain lesion have been proposed: (1) electrochemical production of toxic substances at the electrode, (2) extensive and high-frequency stimulations leading to excitotoxic firing rates, and (3) charge transfer through tissue leading to heating and thermal energy dissipation (Agnew & McCreery, 1987). The Liebetanz et al. (2009) study provided sound reasoning for the damaging mechanism being joule heating rather than the two other mechanisms considered.

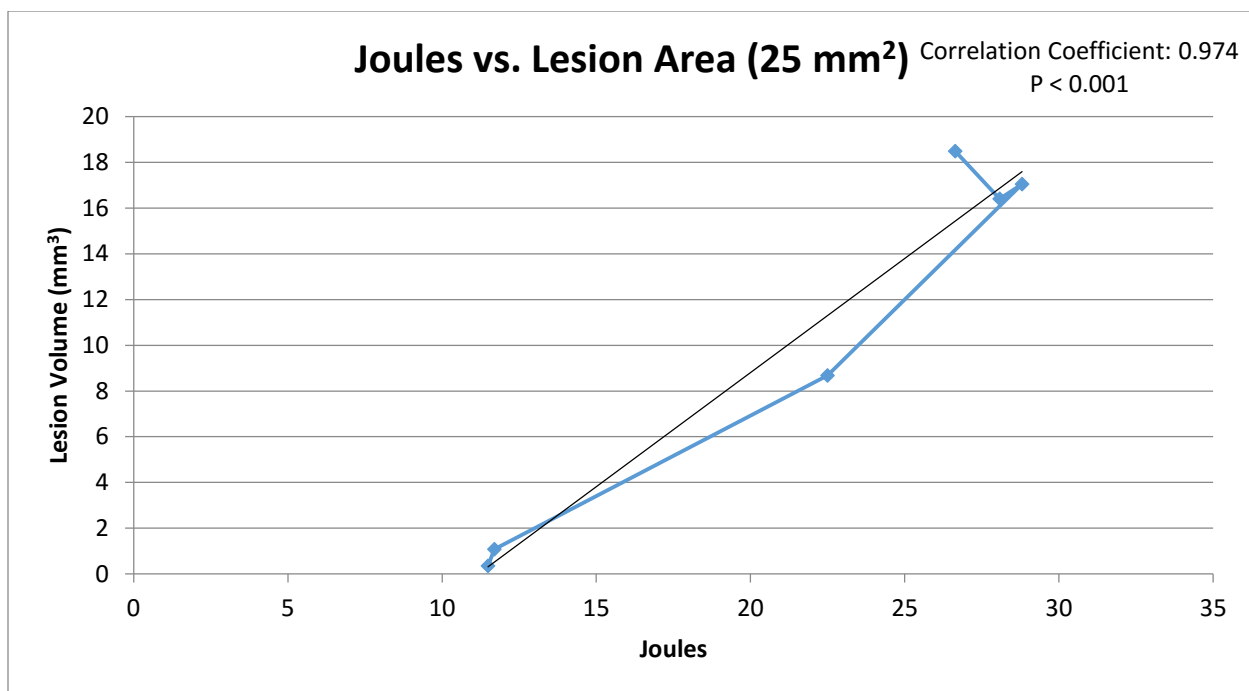
While joule heating would ultimately seem to be involved in our model as well, our study did use anodal tDCS, so excitotoxic firing rates must also be considered. tDCS has been shown to alter stimulated brain regions at subthreshold levels during tDCS (Nitsche & Paulus, 2000), but in our study, it is possible our higher stimulation groups were receiving suprathreshold currents because *in vivo* studies on how tDCS affects a rat's brain have never previously been tested. If the rats in our study were receiving suprathreshold currents, then it is entirely possible some or all of the lesions seen is due to excitotoxic firing rates induced by tDCS. However, joule heating could also be responsible for a significant portion of the brain lesion, so both excitotoxic firing rates and joule heating must be considered as potential, or concurrent, damage mechanisms in our model.

### **Further Discussion of Proposed Lesion Mechanisms**

The Nitsche and Paulus (2000) experiments showed that anodal and cathodal tDCS leads to either an increase or decrease in resting potential, meaning anodal

stimulation increased the amount of millivolts during resting potential and cathodal stimulation decreased the amount of millivolts during resting potential at the region of the brain measured. Voltage, known in electrophysics as electric potential energy, is the ratio of energy (measured in joules) per charge (measured in coulombs), meaning an increase in voltage signals an increase in the amount of energy present on one coulomb of electrons. Conversely, a decrease in voltage signifies a decrease in the amount of energy for one coulomb. This study was increasing the amount of energy per charge in the rat brain through anodal tDCS, likely suprathreshold at higher, lesion producing stimulations; interestingly, an extremely significant positive correlation between the total amount of joules produced from our stimulation and the amount of lesion induced (Figure 10).

The correlation data from this study, when considered from a Hodgkin and Huxley model of action potentials (where action potentials typically occur from increasing mV in a neuron), suggest the amount of energy given to the brain could be responsible for inducing both joule heating as well as excitotoxic firing rates to produce brain lesion in our animals. One could also infer that the amount of energy given to the brain via tDCS could also be responsible for the beneficial effects seen in clinical and experimental studies as well.



**Figure 10:** *Correlating Joules and Lesion Volume. Lesion volume measurements were taken from animals in the 500 and 1,000  $\mu$ A skull stimulation groups. Correlation Coefficient = 0.974,  $P < 0.001$ .*

## V. CONCLUSION

This study was conducted to test aspects of tDCS safety and methodology. The first experiment was conducted to determine a safe anodal tDCS current with our electrode montage that could study physiological changes in learning and memory, and was necessary after visual signs of brain damage occurred at current densities lower than  $142.9 \text{ A/m}^2$  determined by Liebetanz et al.

Our results show brain lesion during a 60 minute anodal tDCS session begins at  $500 \mu\text{A}$ , and the amount of lesion increases as the current is increased. The electrode used in our study had a surface area of  $25 \text{ mm}^2$ , meaning our first lesion was seen at  $20 \text{ A/m}^2$  with our anodal tDCS electrode montage, which is significantly lower than the previously determined lesion threshold of  $142.9 \text{ A/m}^2$ . Additionally, significant differences in areas of lesion in our study occurred under and in front of the electrode, with the lesion area spreading laterally and penetrating deeper into the tissue at higher stimulation intensities. These differences could be due to utilizing different tDCS currents but warrant further study as this study was not intended to be a comparison with the Liebetanz et al. study.

The second goal of this study was to test how the scalp tissue affects the concentration of current reaching the brain by comparing brain lesion from the first experiment to equal stimulation groups but with the anode placed on the scalp.



Computational models predict the scalp tissue will shunt a portion of the current, resulting in a decreased concentration of current reaching the brain.

None of the stimulation groups using a scalp electrode showed brain lesion, suggesting a portion of the current was shunted when the electrode was placed on the scalp; the decreased lesion is in agreement with computational models. Additionally, differences in brain lesion between the skull and scalp electrodes could be due to differences in how the electrons flow to the anode. Lastly, a correlational figure between joules produced as a result of tDCS and brain lesion induced was shown, suggesting joules could be responsible for inducing brain lesion and may be a more reliable method of discussing tDCS dosing.

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